

## VARIATIONS IN THE VENTRAL CILIATURE OF THE CRUSTACEAN SYMBIONT *HYALOPHYSA* (CILIOPHORA, APOSTOMATIDA) FROM MOBILE BAY AND DAUPHIN ISLAND, ALABAMA

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**ABSTRACT** Apostome ciliates are symbiotic organisms whose life cycles are complex and involve specific feeding, divisional, migratory, and phoretic stages. In this study we examined apostome trophonts (the diagnostic stage) from a variety of crustacean hosts in the Mobile Bay and Dauphin Island, Alabama, area. The hosts were grass shrimp (*Palaemonetes pugio* and *P. paludosus*), striped hermit crab (*Clibanarius vittatus*), blue crab (*Callinectes sapidus*), and pink shrimp (*Farfantepenaeus* (= *Penaeus*) *duorarum*). A number of similar but distinct morphotypes of apostomes were present, those corresponding to descriptions of species of *Hyalophysa* as well as variant forms. The morphotypes observed in this study had the following characteristics: variations in the formation of the anterior ventral field of kinetosomes from falciform field 9; variations in the degree to which ciliary row 1 (kinety 1) was separated into 2 segments; and variations in the development of kinety a. A record of the variant morphotypes that do not correspond exactly to an established species should prove useful to biologists attempting to identify apostomes from crustacean molts. We choose not to name the variant forms as new species because they exist as different morphotypes within a population of cells, because some of these types occur in low frequency, and because one of the variant forms changes from one morphotype to another.

### INTRODUCTION

Bradbury (1966) established the genus *Hyalophysa* in 1966 for the organism *H. chattoni*, a common apostomatous ciliate associated with crustaceans in North America. This symbiont spends most of its life cycle encysted on a host such as a shrimp or crab, waiting for a chemical signal to indicate that the host will soon molt. After receiving the signal, the ciliate metamorphoses from a quiescent phoretic cell to a trophont (macrostome) that will excyst upon ecdysis of the crustacean (Figure 1). The trophont then swims to the inside of the exoskeleton and feeds by pinocytosis on the exuvium contained within. Following this single opportunity to feed, the ciliate settles on a substrate, encysts, and produces daughter tomites. The tomites (microstomes) are migratory cells with a non-functional mouth that settle on a crab or shrimp to encyst and begin the cycle again.

Exuviotrophic apostome ciliates are ubiquitous organisms, reported from a wide variety of crustaceans in North America including members of the genera *Pagurus*, *Clibanarius*, *Palaemonetes*, *Cambarus*, *Uca*, *Upogebia*, *Callinectes*, *Sesarma*, *Penaeus*, *Alpheus*, *Lophopanopeus*, *Cancer*, *Panopeus*, and *Carcinides* (Bradbury 1966, Bradbury and Clamp 1973, Grimes 1976, Johnson 1978). Only one report exists in the recent literature that surveys apostomes from a number of hosts from the same locale (Grimes 1976). The present study was undertaken to better understand the apostomes of the Dauphin Island and Mobile Bay region in Alabama by sampling the apostome trophonts feeding in the molts of

a variety of crustaceans. The hosts examined in this study were *Palaemonetes pugio*, *P. paludosus*, *Clibanarius vittatus*, *Callinectes sapidus*, and *Farfantepenaeus* (= *Penaeus*) *duorarum*. Penaeid shrimp names are based on Pérez Fartante and Kensley (1997).

We report many different apostome morphotypes including *H. chattoni* (Bradbury 1966), a number of variants similar to *H. chattoni*, as well as variant forms that do not exactly match published species descriptions. These morphotypes illustrate the variation that occurs in the ciliature within apostome species from one host to another, and provide insights to the transformation from the phoront to the trophont.

### MATERIALS AND METHODS

Grass shrimp (*P. pugio*), blue crabs (*C. sapidus*), and striped hermit crabs (*C. vittatus*) were collected with a dip net or by hand in the airport road marsh, Dauphin Island, Alabama (30°15'N, 88°07'W). Pink shrimp (*F. duorarum*) were collected by throw net from the eastern end of Dauphin Island (30° 15.03' N, 88° 04.60' W), and the grass shrimp *P. paludosus* was collected by dip net at Meaher State Park in Baldwin County, Alabama (30° 39' N, 87° 55' W) between the mouths of the Apalachee and Blakeley rivers. The animals were kept at the main campus of Troy State University in filtered water obtained at the collection site and were fed flaked or pelleted fish food every other day. Their water was changed approximately once a week,

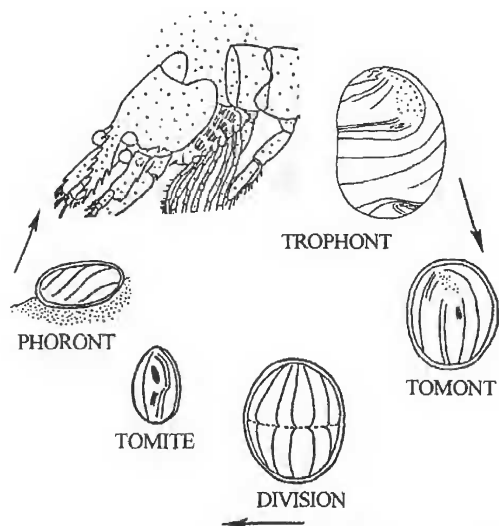


Figure 1. The life cycle of the apostomatous ciliate *Hyalophysa*. Clockwise from the top: trophonts within the exoskeleton, tomonts undergoing division while encysted on the substrate, the swimming infestive tomita, the encysted phoront. Line drawings of the cells are based on silver nitrate impregnation. Adapted from Landers et al. 1996.

Grass shrimp were housed in large groups and only isolated in glass bowls prior to molting. The premolt shrimp were identified by the presence of the developing setae visible under the old exoskeleton in the uropods (Freeman and Bartell 1975). Crabs and prawns were kept in isolation at all times due to the difficulty in identifying premolt organisms. Following ecdysis, the apostomes swimming in the exoskeleton were pipetted directly out of the molt for fixation and silver impregnation.

The ciliates were fixed in 2.5-5% glutaraldehyde for 5-15 minutes. After a thorough washing in distilled water, the cells

were enrobed in warmed gelatin and impregnated with silver nitrate following a modification of the Chatton-Lwoff method (Bradbury and Clamp 1973). Following silver impregnation the cover slips were immersed in cold 70% ethanol, dehydrated, cleared in xylene, and mounted with resin.

## RESULTS

A variety of different apostome morphotypes were observed (Figures 2-10) which had the following 3 characteristics: variations in the dissolution of falciform field 9 (FF9) to form an anterior ventral field of kinetosomes (AVF); variations in the degree to which ciliary row 1 (kinety 1 or K1) was separated into 2 segments; and variations in the development of kinety  $\underline{a}$  ( $K_{\underline{a}}$ ) from FF9. During this study we did not observe variations in the dorsal or the posterior ventral ciliature of the trophont stage, but only differences involving the above named characteristics. Though a gradation of morphotypes exists, the cells that are most representative of the data are illustrated in Figures 2-10. The numbers of each cell type are referenced by the host crustacean in Table 1.

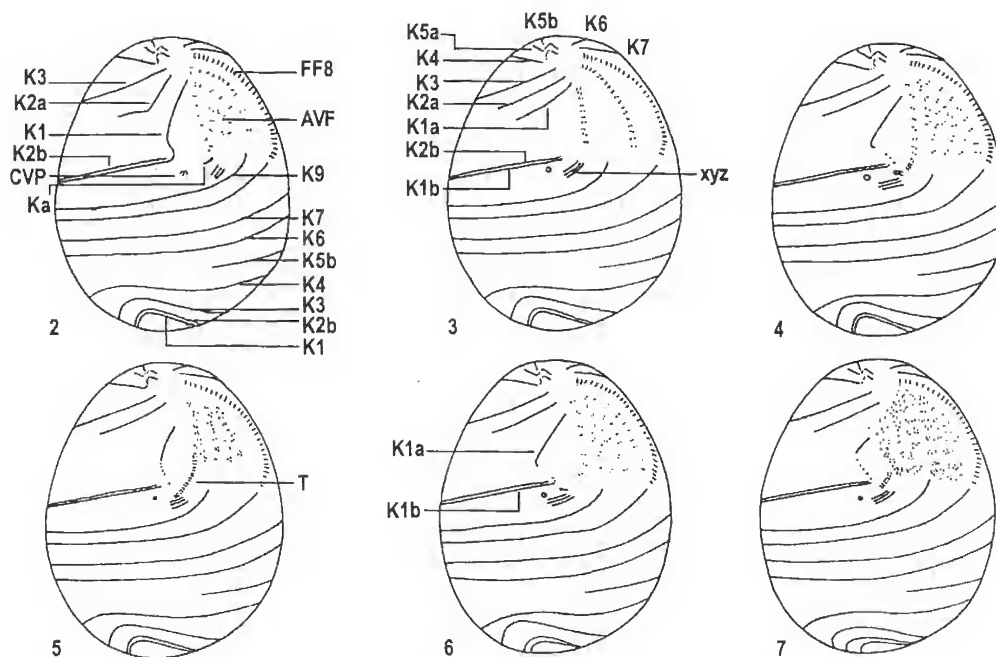
### Apostomes from *Clibanarius vittatus*

Few trophonts (5) were identified from the striped hermit crab, though all exhibited the type ciliature originally described for *H. chattoni* (Figures 2 and 8). This ciliature has been described previously (Bradbury 1966). A brief description of the cell follows: The cell is oval to reniform and measures approximately 55 x 30  $\mu$ m (the size is variable depending upon the amount of ingested food). Nine kineties spiral dextrally around the cell from the anterior to the posterior end. Kinety 1 extends posteriorly along the anterior third of the cell, then bends sharply to the right and continues around the cell. Kinety 2 is divided,

TABLE 1

Listing of all apostome ciliates and their hosts (#observed/#examined). The ciliates are referenced by Figure number from this article and by host. \*Data from morphotype #4 and #5 combined.

Host	Figure #					
	2	3	4	5	6	7
<i>Clibanarius vittatus</i>	5/5					
<i>Callinectes sapidus</i>	1/15		11/15		3/15	
<i>Farfantepenaeus (=Penaeus) duorarum</i>	1/27	7/27	17/27		2/27	
<i>Palaemonetes pugio</i>	18/95	3/95	65/95*	65/95*	1/95	8/95
<i>Palaemonetes paludosus</i>			5/17		12/17	



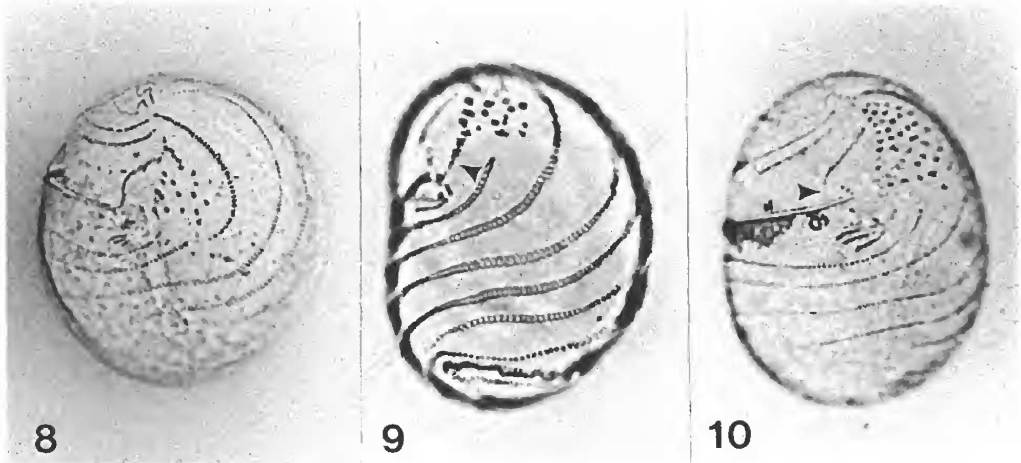
Figures 2-7. The ventral ciliature of trophonts of *Hyalophysa*. Line drawings based on silver nitrate impregnation. Solid lines indicate ciliary rows (kineties). Individual dots represent kinetosomes. K = kinety, CVP = contractile vacuole pore, FF = falciform field, AVF = anterior ventral field, Ka = kinety a, xyz = kineties x, y, and z, T = kinetosomal tail. Figure 2. *Hyalophysa chattoni* type morphology. Figure 3. *H. chattoni* variant with a poorly developed AVF. FF9 has divided into two rows but has not broken into an AVF. Figure 4. *H. chattoni* variant with an altered K1 and AVF. Note the kinetosomal tail, derived from FF9, at the lower right corner of the AVF. Figure 5. *H. chattoni* variant with an altered K1 and AVF. Note the kinetosomal tail, derived from FF9, at the lower right corner of the AVF. Figure 6. *H. chattoni* variant. Note the separation of K1 to form a K1a and K1b and the absence of a kinetosomal tail on the AVF. Figure 7. *H. chattoni* variant. Note the large AVF, kinetosomal tail, and altered K1.

forming a K2a and K2b. Kinety 2a runs along the left of K3. Kinety 4 has a crook at the anterior end and extends around the cell to the posterior. Kinety 5 is divided into K5a, a short Z-shaped fragment, and K5b, which bends around the cell and terminates on the mid-ventral surface. Kinety 6 and K7 spiral from the anterior pole to the posterior pole. The posterior portion of K8 is similar to K6 and K7, but anteriorly it is a double row of kinetosomes termed the Falciform Field (FF8). Kinety 9 parallels K8 on the right. Anteriorly K9 is broken into a field of scattered kinetosomes termed the Anterior Ventral Field (AVF). Three short kineties (x, y, and z) are located to the left of the contractile vacuole pore between K9 and K1. Kinety a is a short kinety located anterior to xyz.

**Apostomes from *Farfantepenaeus* (= *Penaeus*) *duorarum***  
*Hyalophysa* spp. trophonts from *F. duorarum* molts were variable in many respects. In 7 of 27 cells the FF9 did not

break apart to form an AVF but instead formed one to 3 doubled rows of kinetosomes that occupied the area between FF8 and K1a (Figure 3). Additionally, K1 was divided into a K1a and K1b, with K1a completely separated from its lower segment and aligned along the left side of K2a. Kinety a was not observed in these trophonts. This morphology is an intermediate form between *Hyalophysa* and *Gymmodinioides* (Bradbury 1966, Chatton and Lwoff 1935).

The majority (17 of 27) of the trophonts from *F. duorarum* were similar to the *H. chattoni* variant illustrated in Figure 4. In this type, FF9 divided into scattered groups of 2 to 4 kinetosomes to form an AVF and possessed a tail of doubled kinetosomes in the lower right corner, derived from the remnant of FF9. Kinety a was observed in this type. Kinety 1 was either divided into a separate K1a and K1b, separated by a few scattered kinetosomes, or K1a was connected to K1b but appeared to be stretched away from its lower fragment. In addition to this cell type, 2 of



Figures 8-10. Photomicrographs of selected silver-stained apostomes. Figure 8. *Hyalophysa chattoni* type specimen from *Palaemonetes pugio*. The cell is approximately 81  $\mu\text{m}$  wide. Figure 9. *H. chattoni* variant from *P. pugio*. Note the kinetosomal tail (arrowhead). The cell is approximately 59  $\mu\text{m}$  wide. Figure 10. *H. chattoni* variant from *Callinectes sapidus*. Note the break in K1 (arrowhead). The cell is approximately 75  $\mu\text{m}$  wide.

27 cells possessed no tail (Figure 6) and one cell was a type specimen (Figure 2).

#### Apostomes from *Callinectes sapidus*

Most of the trophonts (11 of 15) observed from the blue crab had a morphology similar to the trophont that was most common on *F. duorarum* (Figure 4). K1 was either stretched to the point of separation or was divided into a K1a and K1b and separated by a short gap occupied by 3 to 4 kinetosomes. An AVF was fully formed, with a tail of kinetosomes present in the lower right corner that varied from short (4 kinetosomes) to much more defined (8 kinetosomes). Kinetya was present in these cells, either attached to the tail of kinetosomes or separate from it. In addition to this cell type, 3 cells from *C. sapidus* had no tail (Figure 6) and one was similar to the type morphology of *H. chattoni* (Figure 2).

#### Apostomes from *Palaemonetes pugio*

A large number of cells from *P. pugio* were examined with the majority of the cells (65 of 95) similar to the morphologies illustrated in figures 4 and 5. In these cells a tail of kinetosomes is found at the posterior right corner of the AVF, varying in size from 6 kinetosomes (Figure 4) to 36 (Figure 5). The average number of kinetosomes in the tail was 14 ( $N = 33$ ).  $K_a$  had usually not yet separated from the kinetosomal tail of the AVF. The 30 remaining cells represented a variety of morphologies. Eighteen of the cells were the type morphology (Figure 2), 3 cells had a FF9 that was divided into 2 or 3 fragments rather than an

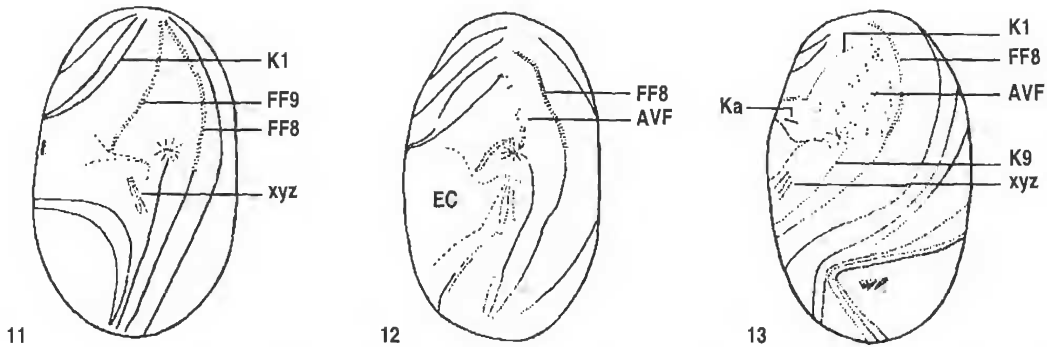
AVF (Figure 3), and one cell had a type AVF but a broken K1 (Figure 6). Finally, 8 cells possessed a large AVF in which individual kinetosomes were spread out into a large shield-shaped field (Figure 7). A tail of kinetosomes was present and K1a was shortened, connected to K1b by scattered kinetosomes. The AVF of this apostome is similar to that of *H. trageri* (Grimes 1976).

#### Apostomes from *Palaemonetes paludosus*

Trophonts from the molts of *P. paludosus* were similar to one of 2 morphologies. Five of 17 cells had a short kinetosomal tail and a bend or break in K1, as seen in apostomes from *C. sapidus*, *P. pugio*, or *F. duorarum* (Figure 4). The remaining cells (12 of 17) had no kinetosomal tail and a separated or bent K1 (Figure 6). Of the last group of cells, 2 had a K1a that did not curve towards K1b but instead was aligned close to K2a. Those 2 cells were most similar to the freshwater apostome *H. bradburyae* (Landers et al. 1996).

#### DISCUSSION

In this study we have demonstrated a number of apostome variants. Particular variants are not restricted to specific species of hosts, but rather, are found in mixed populations on a number of crustaceans. All of the variations result from subtle differences that occur in the cell during the transformation of the phoront stage to the trophont (Figures 11-13). Of all of the changes that take place during this transformation, the formation of the AVF



Figures 11-13. Line drawings illustrating the metamorphosis of the phoront to the trophont during the premolt period on the host (adapted from Landers 1986). Note the formation of the AVF from FF9. K = kinety, FF = falciform field, AVF = anterior ventral field, Ka = kinety a, xyz = kineties x, y, and z, EC = developing extended cytostome.

from FF9 and the bend in K1 are the most variable. The 4 nominal species of *Hyalophysa* are differentiated by characteristics of the AVF and K1, among other features (Bradbury 1966, Bradbury and Clamp 1973, Grimes 1976, Landers et al. 1996). We report variations in the trophont ciliature that involve 3 key characteristics, the AVF, Ka, and K1.

The dissolution of FF9 is a process that occurs normally during the phoretic stage of *Hyalophysa* to form the AVF (Bradbury and Trager 1967). Landers (1986) described this metamorphosis using protargol silver impregnation (see Figures 11-13) and suggested that Ka is a derivative from the posterior fragment of FF9. This hypothesis is confirmed by the present data. Variant forms in which a tail of kinetosomes exists clearly show Ka connected to the posterior tip of the AVF tail.

Kinety 1 is a variable structure among the *Hyalophysa* species. In the *H. chattoni* type morphology, not often seen in this study, K1 has a sharp 90° bend to the right as it extends posteriorly along the right border of the cytostome. This bend is also found in *H. trageri*. In *H. lwoffii* and *H. bradburyae* K1 is divided, though the position of the anterior segment differs. In the present study K1 was most often stretched into either 2 kineties that were barely connected or they were separated by a gap occupied by scattered kinetosomes. Conversely, a wide separation was observed between K1a and K1b in some apostomes from *P. paludosus*, a characteristic more similar to the freshwater form *H. bradburyae* than to *H. chattoni*. A wide separation between K1a and K1b was also present on apostomes with an undeveloped AVF (Figure 3).

The morphotypes described in this report were chosen as representatives to reflect the many variations we observed. One morphotype matches that of a described species (Figure 2) whereas other forms have characteristics

that do not correspond to established species. For example, the cell illustrated in Figure 3 is intermediate between *Gymnodinioides* and *Hyalophysa*. We think this form should currently be considered a variant of *H. chattoni*, and not a species of *Gymnodinioides* because the later genus possesses an unbroken K1, and FF9, if present, is unbroken (Chatton and Lwoff 1935, Bradbury et al. 1996). The cells illustrated in Figures 4 and 5 are similar to *H. chattoni* though in these forms the posterior tip of FF9 has not completed its transformation and remains as a tail of kinetosomes on the ventral surface. The cell in Figure 6 is similar to *H. chattoni* if K1a points posteriorly towards K1b, as illustrated. However, if K1a is more closely aligned next to K2a, the cell is similar to *H. bradburyae*, a freshwater form (note: this form on *P. paludosus* is not surprising, because the shrimp were caught near the Apalachee and Blakeley rivers where a freshwater apostome might be expected). The cell in Figure 7 is similar to the *H. chattoni* variants in Figures 4 and 5 as well as to *H. trageri* (a species known only from the genera *Sesarma* and *Uca*). It is similar to *H. trageri* because of the large shield shaped AVF, but differs from that species in having a kinetosomal tail on the AVF and having a separated K1. At this time we are reluctant to assign the variants illustrated in Figures 2-7 to new taxa because they exist as different morphological types within the same population of cells and because of the low frequency of some of the variant types. Additionally, we have observed that the cells illustrated in Figures 4 and 5 transform into the *H. chattoni* type morphology after feeding has ended (Zimlich, manuscript in preparation), suggesting that some of the variants represent a lag in the development of the *H. chattoni* trophont.

It should be pointed out that some of these variant types are not restricted to Alabama, though they, and not

the established taxa, represent the dominant types from the Mobile Bay area. Neptun (1988) reported the variant illustrated in Figure 5 from *P. pugio* in North Carolina, though it was rarely seen there. Also, the variant described in Figure 3 from *F. duorarum* was found (rarely) in molts of *P. pugio* in North Carolina (S. Neptun, personal communication).

Although different species of apostome trophonts are morphologically distinct, other stages in the life cycle such as the tomont and tomite are remarkably similar to one another (Chatton and Lwoff 1935). In the trophont the cilia are apparently not involved in feeding and can vary in position without affecting the cell. Our data support this hypothesis, since cells of all morphologies bloated normally as they fed within the host's exoskeleton.

Many hypotheses and future experiments can be designed to address the question of why these variants exist and whether the variation in the ventral ciliature has a functional or developmental significance. As the ventral ciliature does not appear to affect the feeding process it is possible that this variation has evolved within the species because there are few selective pressures to restrict the patterning of this ciliature. All of the species of *Hyalophysa* revert to a common morphology as they encyst and produce daughter tomites, suggesting that developmental restraints exist during tomitogenesis that do not allow for as much morphological variation in later stages. There are many factors that could play a role in determining the subtle morphological differences of the trophont's ventral ciliature, such as diet, host animal, water temperature, season, and pollution effects. It is also possible that the morphotypes exist as a result of genetic variations within the population that are not immediately influenced by environmental factors. Future avenues of research are plentiful in this area. For example, apostomes from one host could be used to infect other crustaceans to see if the proportion of the variant types changes with the host. Also, a clonal population of cells could be produced from one trophont and carried through many molt cycles on cleaned shrimp to see if morphological variations are present. Many other experimental variables could be tested in the laboratory to further analyze possible causes of variations in the trophont.

In their historic monograph, Chatton and Lwoff (1935) separated the apostomes into a number of distinct groups based on their diet and life cycles. This study has focused on only one group, the exuviotrophs, whose diet consists of exuvial fluid from crustacean exoskeletons. Earlier reports (Chatton and Lwoff 1935, Bradbury 1966, Grimes 1976, Lindley 1978) leave little doubt that exuviotrophic apostomes exist on probably all crustaceans ranging from

decapods to amphipods to barnacles. While previous reports acknowledge exuviotrophic apostomes, probably of the genus *Hyalophysa*, from the shrimp, *Farfantepenaeus aztecus*, *F. duorarum*, *F. brasiliensis*, *Litopenaeus (=Penaeus) setiferus*, and *L. vannamei*, (Johnson 1978, Lotz and Overstreet 1990), our study confirms the presence of *Hyalophysa chattoni* variants on the pink shrimp, *F. duorarum*, and extends the known record of the genus *Hyalophysa* to a variety of crustacea from the Mobile Bay region. This record establishes the variability present in the apostome population of this region.

Additionally, we have observed apostome trophonts within molts of the mole crab *Emerita* spp. from Dauphin Island but were not able to obtain satisfactory silver stains. Future studies of apostomes will attempt to determine the exuviotroph fauna of crustacea from the high energy beach zones.

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